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### INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

A1 F195/0056	(43) International Publication Date: 17 April 1997 (17.04.97)  (81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE.
	(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE
981, FI	<ol> <li>KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD. SE, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, MW, SD, SZ, UG), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, TE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BI, CF, CG, CI, CM, GA, GN, ML, MR,</li> </ol>
JSEFUL	EXPLOITATION, CARETAKING, ETC. OF A LAND OR WATER
on the ba plied to t condition	, tending, determining of the condition of a land or water area etc., in sis of a sample taken from the soil or water and, on the basis of the the area. The procedure is characterized in that microbial growth in the of the area is estimated on the basis of said microbial growth and the surces or equivalent to be applied to the area. The invention also relates
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PROCEDURE AND IMPLEMENTS FOR USEFUL EXPLOITATION, CARETAKING, ETC. OF A LAND OR WATER AREA

The present invention relates to a procedure for useful exploitation, tending, determining the condition etc. of a land or water area, as defined in claim 1. Moreover, the invention relates to implements for the implementation of the procedure of the invention, as defined in claim 10.

'Useful exploitation, tending, determining the condition' of an area here refers to any productive or environmental activity concerning the area, such as agriculture, gardening or forestry/afforestation; fertilization, condition monitoring and/or amelioration of an area; or evaluating, monitoring or improving the overall condition of a land or water area e.g. in conjunction with official environmental surveillance, planning or research or commercial exploitation etc. of an area, yet without being restricted to these examples.

In conjunction with productive activities concerning a land area, e.g. agriculture, forestry or afforestation, a known practice is to examine the condition of the soil to provide a basis for the planning of actions to be taken concerning the area, e.g. to estimate the need for fertilization.

In prior art, the condition of a land area is estimated by carrying out a chemical analysis. According to the current methods, this involves taking one or more samples of the soil and sending them to a research laboratory for analysis. Before the chemical analysis is carried out, the samples are dried and powdered.

The current methods of analysis have the drawback that, even though they are designed for the estimation of the condition of the soil in respect of nutrients, chemical methods are still not adequate for specifically determining the portion of the nutrient

content of the soil that is useful for plants, because such methods cannot be used to simulate the absorption of nutrients by plant roots, which varies considerably depending on external conditions. Therefore, with chemical methods it is only possible to determine a certain soluble portion of the nutrients. In practice, the properties affecting nutrient supply, such as acidity, water content, compactness etc. of the soil, show such great variations that it is sometimes diffi-10 cult to establish a clear dependence between the content of soluble nutrients in the soil and the nutrient supply of plants. Furthermore, the drying and crushing of the samples changes the properties of natural soil, including the solubility of nutrients, and these changes are different in different types of soil. The abo-15 ve-mentioned factors to some extent reduce the practical applicability of the results.

Environmental conditions, such as the degree of pollutedness, e.g. the occurrence of heavy metals, have also so far been established and monitored on the basis of chemical analyses.

Current methods of investigation are therefore based on only determining the amounts of compounds and elements in samples. However, the microfauna has a substantial effect on the soil and as well as the growth of plants and their nutrient absorption. Microbes act as a connection between the animate and inanimate parts of the ecological system. They decompose dead organic matter, thereby binding energy and nutrients from plant and animal remains in themselves. Due to decomposition, plants are able to utilize the nutrients in the soil. Microbes also affect the composition of vegetation.

An example of microbes are fungi, which make 35 up most of the biomass. For instance there are fungi which live in symbiosis with plant roots, forming a mycorrhiza. For plants, mycorrhiza infection is very

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important, even indispensable. The mycorrhiza contributes towards a more efficient absorption of nutrients by a plant, maintains its health, protects against stress factors, improves the composition of the soil and reduces erosion and denudation. Other soil microbes and their activities also have a great importance for plants.

Unnaturally fast acidification of the soil and intensive use of fertilizers or pesticides, herbicides and plant disease fighting agents have a negative effect on the balance of nature, possibly resulting in unbalanced conditions between different microbes, unnatural populations, or complete disappearance of some species of microbes. For example, it has been established that certain microbes and root fungi are very sensitive to fertilizers or cannot tolerate them at all. An unbalance between microbial populations naturally has a detrimental effect on the condition of the soil and the plants living in it.

The object of the present invention is to eliminate the drawbacks described above.

A specific object of the present invention is to present a new type of procedure for useful exploitation, tending, determining the condition of a land or water area, which allows a completely different approach in estimating the condition of a land or water area e.g. for the planning and implementation of productive activities in the area, such as farming, forestry or afforestation, and which allows e.g. productive activities concerning the area to be planned and implemented in a manner that takes the natural condition of the area into account.

A further object of the present invention is to present a new type of procedure for useful exploitation, tending, determining the condition of a land or water area, in which procedure a sample can be used repeatedly to estimate the condition of the area under

investigation without a need to send the sample to a research laboratory.

Another object of the present invention is to present means that allow the procedure of the invention to be implemented in simple stages and repeatedly and which can be delivered as an easy-to-use, moderate-prized commercial assembly for each application.

As for the features characteristic of the invention, reference is made to the claims.

10 In the procedure of the invention, one or more samples taken from the land or water area concerned are examined to determine the microbial growth them, the condition of the area is estimated based on said growth, and the growth results obtained are used 15 in the planning of measures or equivalent to be taken concerning the area. The microbial growth can be determined by applying the practices generally known in microbiology, for instance by keeping a sample for a given time in conditions that allow the growth of the microbe under consideration to form a possible micro-20 bial population. In the estimation of the condition of the area on the basis of the growth results and in the planning of further measures or equivalent concerning the area, it is possible to use e.g. existing informa-25 tion about the microbe/microbes under investigation or an instruction file provided for the procedure of the invention

The term 'microbe' as used in this context refers generally to any microgerms, e.g. bacteria, fungi, yeasts, algae, spores, primordial germ cells, viruses, etc. that may be present in the area under investigation. The microbe to be investigated can be selected from among one or more microbe species relevant for the area under study and the objective of investigation in each case, e.g. a useful or a detrimental species for plants.

As is known in the field of microbiology,

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'microbe population' refers to individual cells, colonies, mycelial filaments, including the mycorrhiza in the case of root fungi, etc., whose amounts, quality and/or relationships to each other can be determined.

So far, microbial growth estimations have not been used as an aid e.g. in the planning and implementation of productive activities concerning a land or water area as mentioned above. Estimations based on a chemical analysis as used before do not give any indications about the microbiological condition of a land or water area. For example, in conjunction with agriculture and forestry/afforestation, plans based on such estimations may instead result in incorrect cultivation/plantation, fertilization etc. with regard to the microbial balance of the area, and this may lead to unfavourable consequences in respect of the growth and health of plants.

In the procedure of the invention, useful exploitation, tending, determination of the condition etc. of an area are planned and implemented on the basis of a specified microbial growth, so the procedure makes it possible to implement e.g. farming and forestry in a manner conducive to better conservation and exploitation of nature. Furthermore, due to the estimation of microbial growth as used in the invention, the procedure allows, among other things, a better estimation of the absorption of nutrients by plants and of the proportion of nutrients in the soil that is useful for plants.

Thus, the procedure of the invention can be used as an alternative method or as a complementary method to the chemical methods of estimation so far used in the planning of measures or equivalent concerning a land or water area.

According to a preferred embodiment, the procedure of the invention is used in the planning of cultivation/growing programmes, soil improvement or

fertilization in agriculture or forestry and, if necessary, in the monitoring of the implementation of measures concerning an area, e.g. for monitoring the effects of fertilization or the condition of the soil during the growing season, allowing the implementation to be controlled on the basis of the monitoring results obtained.

In an additional embodiment of the procedure of the invention, seeds/seedlings are germinated/grown 0 in a sample taken from an area under investigation in conjunction with the estimation of microbial growth in order to select on the basis of the germination/growing results a plant species or variety or a corresponding seedling population suited for the area.

- 15 Further, in another additional embodiment, the growth results obtained from the microbial growth estimation of the invention are compared with the microbial population of the substratum of seedlings to be planted in order to investigate the suitability of the plants for the area, and/or the microbial population of the sample or substantially a population corresponding to the
- le or substantially a population corresponding to the microbial population of the sample is used to infect the roots of the seedlings to be planted prior to planting to obtain a seedling population suited for the area under investigation.

The procedure is also excellently suited for the monitoring of the condition of a land or water area e.g. in conjunction with environmental supervisi-

on.

- 30 The manner of implementation of the procedure of the invention is not limited in any way. Preferably the procedure is implemented using implements as provided by the invention, which comprise
- (a) at least one sample container for each microbe or group of microbes under investigation, said sample container containing a culture medium suitable for the microbe or group of microbes;

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- (b) a culture space for the sample containers to create conditions favourable for the growth of each microbe or group of microbes under investigation; and optionally
- 5 (c) a model file in which growth descriptions for each microbe or group of microbes under investigation have been collected beforehand to allow comparisons between growth samples and which is provided with a set of instructions for estimating the condition of the area on the basis of the growth results obtained.

The implements of the invention can be fitted to correspond to the area concerned and to the target of investigation. The implements may be available separately or they may be available as a commercial assembly.

The sample containers (a) are intended for samples taken from the area under investigation for the determination of the occurrence, amount and/or mutual relationships of one or more microbes e.g. by methods known in microbiology. The sample containers may be like those generally used in microbiology.

The composition of the culture medium can be so selected that it will restrict the growth of undesirable microbes and microbes having no importance with respect to the result.

The culture space (b) may be any space capable of accommodating the sample containers during the growing of the microbes under investigation. A preferred culture space is a culture box known in itself, which permits regulation of the conditions in it, such as temperature and/or light.

In addition, the implements of the invention preferably comprise a model file (c). It contains microbe descriptions which may be based on a previously collected verbal description and/or pictorial representation of a corresponding microbial growth. To estimate the biological condition of the area, the

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microbial growth as determined from the sample can be compared with the model file with its set of instructions. The file preferably contains instructions for the interpretation of the results according to the needs of the relevant investigation objectives. The purpose of such a model file is to make it easier to estimate the condition of the area on the basis of the microbial growth produced.

When the implements include a model file composed beforehand for the application in question, the
culture mediums and growth conditions in the sample
containers preferably correspond to the culture mediums of cultures collected in the model file for the
microbe under investigation and to the conditions used

15 for those cultures.

According to a specially preferred embodiment, the model file can be provided with a set of instructions for the planning of actions possibly to be carried out on the area on the basis of the growth results obtained according to the needs of the relevant investigation objectives.

Alternatively, the microbial growth samples can be sent to a research laboratory for investigation.

25 According to a preferred embodiment, the implements of the invention preferably comprise a measuring device for pH measurement known in itself to allow the pH-value of the sample to be determined, in which case the pH-value of the sample can be used together with microbial growth estimation to obtain an assessment of the condition of the area.

As compared with prior-art equipment for chemical analyses, the implements of the invention are of moderate cost and easy to use. Moreover, the implements allow the procedure of the invention to be carried out repeatedly and reliably. The implements of the invention make it possible e.g. for a producer,

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such as a farmer, to carry out the procedure him/herself.

In the following, the invention is described by referring to the drawings, in which

Fig. 1 presents an embodiment of the implements of the invention, and

Fig. 2 presents another embodiment of the implements of the invention.

As shown in Fig. 1 and 2, the implements of 10 the invention comprise sample containers 1a, 1b, 1c, ld. The sample containers are provided with a culture medium, liquid or solid, that allows the growth of the microbe under investigation. A sample container may be designed for one or more microbes. If necessary, the sample containers can be divided into compartments e.g. using partitions 1b, 1c, 1d, and each compartment may contain a different culture medium. In this way, it is possible to obtain an overall analysis or a de-

sired complex of analyses of the same sample. 20 Further, the implements of the invention com-

prise a culture box 2, whose interior temperature is preferably adjustable and which is provided with a light source 5 to produce light in the box when necessary. Moreover, the implements preferably comprise a model file 3 in which growth descriptions for corresponding microbes have been collected beforehand to allow comparisons and which is preferably provided with a set of instructions for estimating the condition of the area and preparing cultivation plans, soil improvement programmes and/or fertilization plans on the basis of the growth results obtained.

Furthermore, the implements may comprise pH paper for the determination of the pH-value of a sample for the estimation of the condition of the area.

3.5 The implements may also comprise general instructions for the use of the implements, instructions relating to growth conditions.

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In the procedure of the invention, a representative sample is taken from a field land area to be analysed. The sample is preferably in a natural condition, but if necessary it can be treated, e.g. elutriated, without destroying the microbes in it. A sediment can be prepared using e.g. a ringer solution or a saline solution. The sample is brought onto a culture substrate in a sample container la, lb, lc, ld without touching the sample. The sample container is closed and placed in a culture box 2 where the conditions (light, temperature and/or humidity) preferably correspond to those used in the model file cultures.

Upon the lapse of a given time, the sample container 1 is taken out and the growth formed on the culture substrate is compared with corresponding culture descriptions in the model file 3 included in the implements, e.g. with respect to the amount, quality and/or mutual relationships of microbial growth. The model file 3 preferably also contains instructions for estimating the condition of the area and for further measures to be taken. Thus, the person carrying out the estimation has the instructions available for interpretation of the results and planning of further measures.

In the following examples, an embodiment of the implements and of the procedure of the invention is described by referring to Fig. 1 and 2.

### Example 1:

30 As an example of the implements of the invention, implements for examining a field/garden area are presented. The implements comprise:

(a) sample containers la containing a culture medium that allows the growth of an arbuscular mycorr-

35 hiza;

(b) a culture box in which it is possible to create conditions favourable for the growth of an ar-

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buscular mycorrhiza;

(c) a model file in which growth descriptions for a corresponding mycorrhiza have been collected beforehand and which contains a set of instructions for estimating the condition of the area and preparing cultivation plans, soil improvement programmes and/or fertilization plans on the basis of the growth results obtained.

 $\,$  (d) pH paper for determining the pH-value of 10  $\,$  the sample.

The culture medium for an arbuscular mycorrhiza preferably contains the following main nutrients: N, P, K, S, Ca, Mg, Fe; micronutrients: B, Mo, Mn, Co, Zn, Cu, Cl, I; saccharose as a carbohydrate energy source; vitamins: inositol, thiamin, etc.; growth regulators: auxins (IAA, IBA, NAA), cytokinins (kinetin, BAP, zeatin, 2-4-D), gibberellins (and antigibberellins), coconut milk, casein hydrolysate; active carbon; and agar and gelrite as gel forming substances. The culture mediums and growth conditions correspond to those used in the model file.

To allow the formation of a mycorrhiza, roots are provided in the sample container la. The roots can be obtained from a sample, a parent plant and/or seeds brought into the sample container. To permit germination/growth of the seeds/plants, the culture box 2 contains a light source 5. If desired, it is possible to use seeds/seedlings of different species/varieties as seeds/parent plant to perform a germination/growth test simultaneously with the microbial culture. A germination test can be used to establish the suitability of different plant species or varieties or equivalent for a given area. A germination test can also be carried out without a microbial estimation.

35 When seeds/plants are germinated/grown in a sample container la, the container la is preferably provided with a cupola-shaped cover, e.g. a hothouse

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dome 4.

The implements may also include sample containers la containing a culture medium allowing the growth of the Rhizobium microbe and/or other microbes useful for agriculture. Alternatively, the sample containers lb, lc, ld can be divided with partitions into compartments for different microbes, and each compartment may contain a different culture medium. Overall growth can also be determined. In this case the model file has been complemented by taking all the microbes under investigation into account.

In general, the implements of the invention can be implemented by applying general practices associated with microbiology; for example, the implements may comprise sample containers for control and/or reference samples.

#### Example 2:

The procedure of the invention for investiga-20 ting a field land area for cultivation and/or fertilization plans is carried out using implements as described in Example 1.

- 1. A representative sample (including roots) is taken from the field land area to be investigated.
- 2. The sample is brought onto a culture substrate in a sample container 1 provided with a hothouse dome 4 without touching the sample. A parent plant or plant seeds are brought onto the culture substrate to produce roots and, if desired, to perform a germination test at the same time.
- 3. The sample container 1 is closed and provided with information relating to the object of investigation.
- 4. The sample container 1 is placed in a cul-35 ture box 2 in conditions (light, temperature, humidity according to known practice) favourable for an arbuscular mycorrhiza, corresponding to the conditions

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used in model file cultures.

5. Upon the lapse of a given time, the sample container 1 is taken out and the growth that has appeared on the culture substrate is compared with corresponding culture descriptions in the model file 3 comprised in the implements, e.g. as regards the amount, quality and/or mutual relationships of microbial growth. The model file contains instructions for the estimation of the condition of the area and for further measures.

In addition to microbial growth, the pH of the area under investigation is also determined e.g. from a sediment obtained from a small amount of sample material and the pH-value is used together with the microbial analyses for the estimation of the condition of the area.

The infection of roots is an indication of the amount and quality of fungi in the soil under investigation. By determining the growth of a mycorrhiza it is possible to estimate e.g. the nutrient absorption of plants and/or excessive use of fertilizers (certain fertilizers may destroy the mycorrhiza).

Further, according to an embodiment, since e.g. the plants of forest trees are grown e.g. in nurseries on different substrates as compared with the area under investigation, the microbial analysis of the invention can be used to establish whether an intended plantation area has the same/suitable microbe population as the plants have in the nursery. Thus, if necessary, the roots of the plants can be infected with a microbe population corresponding to that in the sample or with the microbe population of the sample prior to planting. If desirable, the infection can also be performed without a microbial analysis.

The procedure and implements described in the foregoing can also be applied within the sphere of protection of the invention e.g. for establishing the

health of the soil in an area under investigation or of a water system, for the investigation of wood land and bioenergy or for the measurement of pollution in the soil and in water systems. As for microbial analyses and instructions, the applications may be implemented by making use of the techniques known in microbiology.

Further, the procedure can also be implemented by bringing into the sample microbes alien to the area under investigation, in which case the growth or destruction of such microbes in the sample will act as an indicator of the condition of the area.

The examples are only intended to provide an illustration of the invention, without limiting it in 15 any way.

CLAIMS

- 1. Procedure for useful exploitation, tending, or determining of the condition of a land or water area, in which procedure the condition of the area is estimated on the basis of a sample taken from the soil or water and, on the basis of the estimate obtained, measures or equivalent are possibly applied to the area, characterized in that microbial growth in the sample taken from the area concerned is determined, the condition of the area is estimated on the basis of said microbial growth and the microbial growth results obtained are used in the planning of measures or equivalent to be applied to the area.
- 2. Procedure as defined in claim 1, cha15 racterized in that the microbial growth as determined from the sample is compared with a model file
  composed beforehand, presenting growth descriptions
  for the corresponding microbe, to estimate the biological condition of the area, and the measures possibly
  20 to be applied to the area are planned on the basis of
  the results obtained.
  - 3. Procedure as defined in claim 1 or 2, characterized in that the sample is kept for a given time in conditions allowing the growth of the microbe under investigation to produce a possible population of the microbe.
  - 4. Procedure as defined in claim 3, characterized in that the sample is kept in growth conditions corresponding to the growth conditions of cultures of the corresponding microbe collected in the model file.
    - 5. Procedure as defined in any one of claims 1-4, characterized in that the pH of the sample is determined for the estimation of the condition of the area.

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growth analysis is used in the planning and monitoring of implementation of measures associated with agriculture, gardening, forestry or in the monitoring of the condition of a land or water area or in the planning of its improvement.

- 7. Procedure as defined in any one of claims 1 6, characterized in that at least one sample is taken from a field land area and the growth of root fungi is determined e.g. as the growth of a mycorrhiza, and possibly the growth of the Rhizobium microbe is determined; the population produced is compared with a model file composed of descriptions of corresponding microbe populations and the results obtained are used as a basis for cultivation plans, soil improvement programmes, fertilization plans etc.
- 8. Procedure as defined in any one of claims 1 7, characterized in that in conjunction with the determination of microbial growth in the sample, seeds/plants are germinated/grown in the sample to allow the selection of a plant species or variety or a corresponding seedling population suited for the area on the basis of the germination/growth results obtained.
- 9. Procedure as defined in any one of claims 25  $\,$  1 8, characterized in that
  - (a) the microbial growth as determined from a sample taken from the area under investigation is compared with the microbial population in the substratum for seedlings to be planted in order to establish the suitability of the plants to the area; and/or
  - (b) the microbial population of the sample or substantially a microbial population corresponding to the microbial population of the sample is used to infect the roots of the seedlings to be planted prior to planting to obtain a seedling population suited for the area under investigation.
    - 10. Implements for use in the procedure of

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the invention, in which the condition of a land or water area is estimated on the basis of a sample taken from the soil or water and, based on the estimate obtained, measures or equivalent are applied to the area, characterized in that the implements comprise

- (a) at least one sample container for each microbe or group of microbes under investigation, said sample container containing a culture medium suitable for the microbe or group of microbes;
- (b) a culture space for the sample containers to create conditions favourable for the growth of each microbe or group of microbes under investigation; and optionally
- (c) a model file in which growth descriptions for each microbe or group of microbes under investigation have been collected beforehand to allow comparisons between growth samples and which is provided with a set of instructions for estimating the condition of the area on the basis of the growth results obtained.
  - 11. Implements as defined in claim 10, characterized in that the model file is provided with a set of instructions for the planning of actions possibly to be carried out on the area on the basis of the growth results obtained.
- 12. Implements as defined in claim 10 or 11, characterized in that the culture mediums correspond to the culture mediums used in the cultures of the microbe under investigation that are included in the model file and the conditions in the culture space can be made to correspond to the conditions of the cultures of the microbe under investigation that are included in the model file.
- 13. Implements as defined in claim 10 or 12, 35 characterized in that the implements comprise a pH measuring device for the determination of the pHvalue of the sample.

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- 14. Implements as defined in claim 10 or 13, characterized in that the implements are consistent with the object under investigation and the objective of investigation in each case.
- 5 15. Implements as defined in claim 10 or 14, characterized in that the implements are intended for use in the planning of measures related to agriculture, gardening or forestry/afforestation or for the establishment, monitoring or improvement of the condition of a land or water area.
  - 16. Implements as defined in any one of claims 10 15, characterized in that the implements are fitted for the planning of the utilisation of a field land area intended for agricultural purposes and the implements comprise
  - (a) one or more sample containers containing a culture medium that allows the growth of an arbuscule, e.g. a mycorrhiza, and/or a culture medium that allows the growth of the Rhizobium microbe.
- 20 (b) a culture box in which the conditions can be made favourable for the microbes to be grown;
  - (c) a model file which contains a collection of growth descriptions for corresponding microbes and a set of instructions for estimating the condition of the area under investigation and for preparing cultivation plans, soil improvement programmes etc. on the basis of the growth results obtained.

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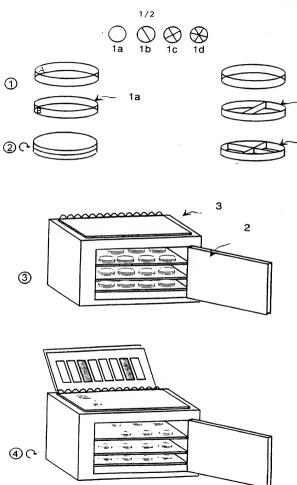


Fig 1

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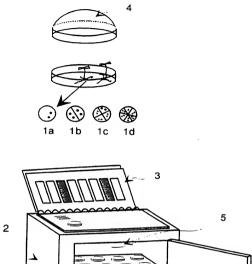


Fig 2

### INTERNATIONAL SEARCH REPORT

International application No.

			PCT/FI 95/0	0567				
A. CLAS	SIFICATION OF SUBJECT MATTER							
IPC6: C	120 1/64, C120 1/02 o International Patent Classification (IPC) or to both	national classification an	d IPC					
B. FIELDS SEARCHED								
Minimum d	ocumentation searched (classification system followed	by classification symbols	)					
IPC6: C								
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	I,NO classes as above							
Electronic d	ata base consulted during the international search (nam	ne of data base and, whe	re practicable, searci	terms used)				
BIOSIS,	CA, WPI, USPATFULL							
C. DOCU	MENTS CONSIDERED TO BE RELEVANT							
Category*	Citation of document, with indication, where a	Relevant to claim No.						
х	US 3236600 A (W. SHRIMPTON ET A 22 February 1966 (22.02.66)	1						
A	A DE 4027284 A1 (ENDERS, JOSEF), 5 March 1992 (05.03.92)							
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	Patent Office S-102 42 STOCKHOLM	0						
	5-102 42 STOCKHOLM	Carolina Gómez Lagerlöf						

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Information on patent family members

International application No. PCT/FI 95/00567

Form PCT/ISA/210 (patent family annex) (July 1992)